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Studies on Thermal Death Points of Yeasts

STUDIES ON THERMAL DEATH POINTS OF YEASTS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY MIRIAM DOUGHERTY

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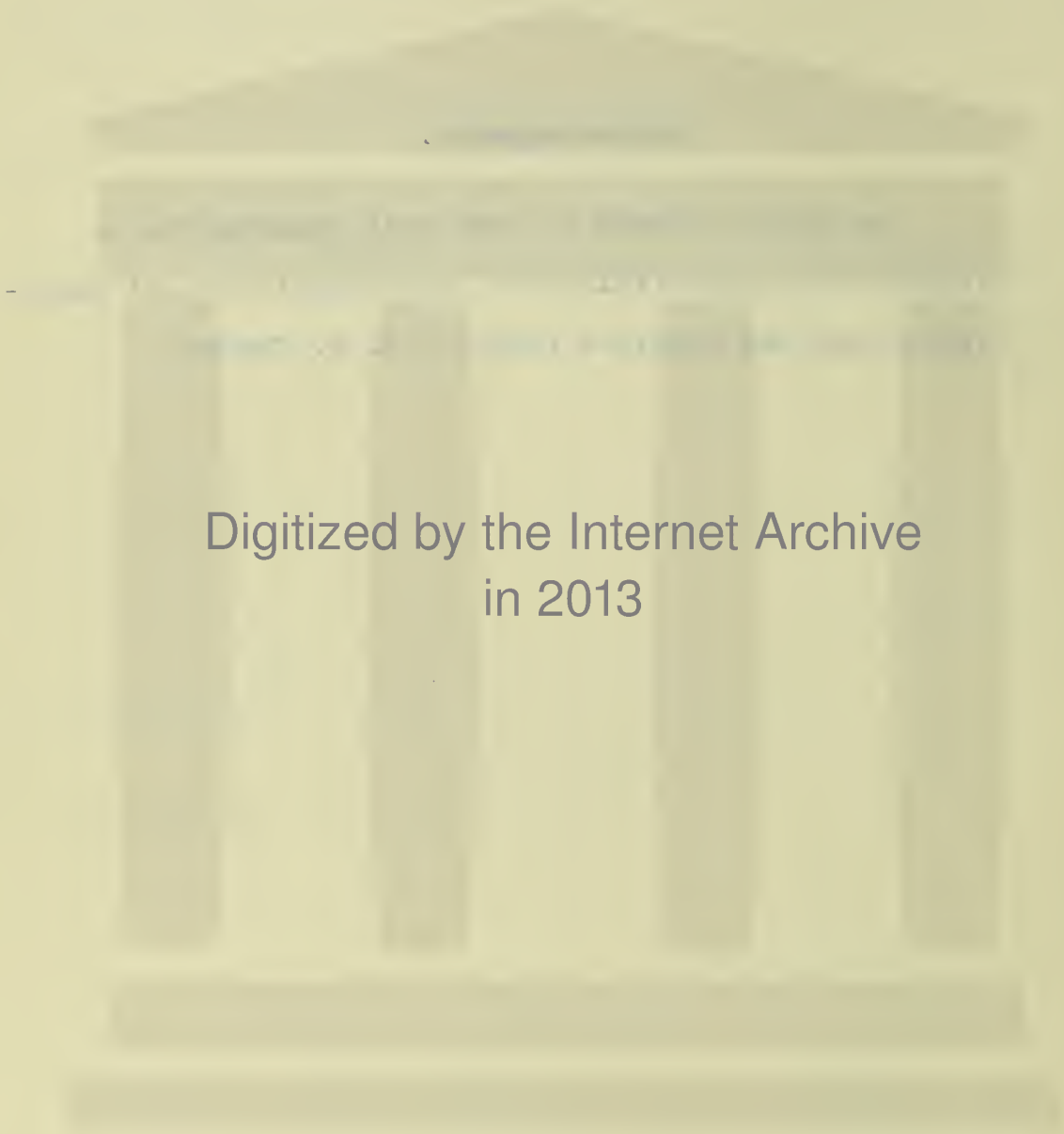


TABLE OF CONTENTS.

I. Introduction.

The reaction of microörganisms to different ranges of temperature constitutes an interesting theoretical as well as very practical characteristic of microörganisms. Many of the endeavors of man to prevent food spoilage rest on efforts to keep the foods at temperatures which are unfavorable for various microörganisms. Refrigeration is practiced extensively for such foods as meat, eggs, vegetables, etc. More important, however, seems tobe the application of heat. This implies the fact that certain temperatures destroy the life of microörganisms. The temperature at which these organisms die, is termed the "thermal death point". The Society of American Bacteriologists states that the organism shall be held for ten minutes at the various temperatures before testing their resistance. Much data is available for the large groups with the exception of the yeasts. This study is limited to that group.

II. Relation of Heat to Life in General.

(a) Effect of Temperatures on Animals.

All organisms of both the plant and animal kingdoms have their maximum, minimum and optimum temperatures, the range of growing or living temperature depending to a great extent on the species and on the ancestral history of the individual. Many investigators have carried out experiments with the effects of varying temperatures and have observed numerous interesting reactions and variations.

When an ameba comes into contact with a hot area, it responds negatively -- that is, it moves in another direction. Mendelssohn (1895) found that a similar response is evinced by Paramecium when it is subjected to extreme heat or cold. This action seems to be caused by change of conditions, for Paramecia soon become acclimatized except when they are under conditions of such intense action that they are quickly destroyed. For example when they are subjected to a temperature of 38°C they move quickly backward and forward until they die. Passage from optimum temperature to either hot or cold always induces a negative reaction -- heat increasing and cold reducing the speed -- while a change leading toward the optimum does not cause this reaction. Infusoria, in general, act the same toward temperature changes as does Paramecium (Jennings, 1904).

In 1903, Mast found that when Hydra is subjected to a temperature of 31°C it loosens its foot-hold and moves either toward

or away from the stimulation, the change being merely one of position. A decrease in temperature, however, does not cause the Hydra to change its position. It merely causes the animal to become sluggish.

When Planaria enters a hot or cold region, it waves its head and attempts to return to the region of former conditions; but if the temperature is raised uniformly, a rapid gliding follows and, as the temperature increases, the animal makes rapid, violent contractions, forms a spiral, rolls the two ends under the body and dies.

That protozoa can be acclimatized to 70°C by raising the temperature gradually thru a period of several years, has been shown by Dallinger (1880). Jacobs (1919) found that Paramecium stands 44 times the theoretical fatal injury when subjected to gradually increasing temperatures, although starfish larvae show practically no resistance or acclimatization. Davenport and Castle (1895) found in hot springs that protozoa live at 60°C, green algae at 70°C, and blue green algae at 80°C, while various metazoa live at 45°C and over. They also found that tadpoles can be acclimatized to a temperature of 43°C, by exposing them to 24.5°C for four weeks. They concluded that acclimatization is due to decreased water content of the tissues. On the other hand Vernon (1899) states that the effects of heat are not accounted for by the amount of water in the tissues. He found that in the ovum of Strongylocentrotus, the death temperature increases with the increase in percentage of water in the tissues. Mayer concluded

that death is probably due to accumulation of acid (H_2CO_3) in the tissues. He found that corals are easily killed by smothering in mud, and by heating, the cause probably being due to asphyxiation. Behre (1918) believed that acclimatization to temperature changes the rate of metabolism.

In larger animals the stage of development is important. Davy found eggs more resistant than young fish. At 87°F both young and adult fish were dead while the eggs lived to hatch into normal, vigorous larvae. Loeb and Westenays (1912) observed that the resistance of fish to high temperature varies directly with the concentration of the salts of sodium, potassium and calcium in sea water. The effect does not seem to be due to osmosis but to some unknown action of the salts present. They found too, that an intermittent exposure to a high temperature is just as effective an immunizing factor as prolonged exposure, and that fish subjected to 27°C for thirty hours are immunized to 35°C and retain this immunity after being replaced for a time in a temperature of 10°C or lower. Wells (1914) found that the larger the fish of a given species, the higher the resistance and that this resistance is highest just before the breeding season and lowest immediately afterward.

Shelford (1916) found that organisms from shallow water can withstand a longer exposure to a given high temperature than those from deep water.

That there is a definite temperature coefficient for the duration of life in the fruit fly (Drosophila) has been proved by Loeb, Jacques and Northrop (1917). This temperature coefficient is approximately identical with that for the duration of

life in the larval and pupal stage between 15° and 25°C, i. e. within the limits where development is normal. Since these flies were raised under aseptic conditions, the observations on the temperature coefficient for the duration of life suggest that this duration is determined by the production of a substance leading to old age and natural death, or by the destruction of a substance or substances which normally prevent old age and natural death.

A strain of Drosophila in which one or more legs were reduplicated was discovered by Hoge (1915). Under ordinary cultural conditions only about 10% of the individuals from a pure reduplicated mating show this condition. It was later discovered that by subjecting the eggs to a temperature of 9°-10°C the percentage of offspring showing this characteristic can be raised to practically 100, and that exposure ofthe eggs to cold produces the greatest percentage of reduplication of legs in the imago.

Krafka (1919) observed the effect of temperature upon facet number in the bar-eyed mutant of Drosophila and found that the difference in the number of facets per Centigrade degree is greatest at the low temperatures and least at the high temperatures, the difference in the number varying with the mean. The per cent of change in the mean facet number is greatest at the lower (15°C-17.5°C.) and higher (29°C-31°C.) temperatures and least at the intermediate temperatures. Temperature seems to be a factor in determining facet number only during a relatively short period in larval development. This period at 27°C comes between the end of the third and end of the fourth day. The number of facets and length of the immature stage (eggs, larva and pupa) appear related when the total development takes place at a constant temperature.

The amount of pigmentation of the adult was observed by Tower (1906) to be affected by subjecting larvae of Leptinotarsa decemlineata to various temperatures. He found that an increase from the mean temperature range (22.5°C.) of the species has practically the same effect as a decrease. He obtained an increase in melanism with a temperature as low as 16°C and as high as 28°C followed by a decrease to albinism beyond these temperatures. Shelford (1917) observed that there is a tendency toward melanism with an increase in temperature, caused by reduction in size of the unpigmented areas on the elytra of the tiger beetles.

Another instance of the effect of temperature is cited by Vernon (1902). He found that in the majority of cases temperature acts directly on all the cells composing the tissues, promoting or retarding their growth, and producing a permanent effect on the organism as a whole.

Higginbottom observed that with frog ova a difference of 5°C in temperature more than trebles the period of development. For the growth of tadpoles tails, Lillie amd Knowlton found the optimum to be 30°C the rate of increase in length being 10.6 times greater than at 10°C. At 31° to 34.9°C the rate is only nine times greater.

Interesting experiments have been carried out with the effect of temperature on wing colors and markings of Lepidoptera. It was once thought that Venessa levana and V. prorsa were of different species, but it is known now that they are of the same species. V. levana, emerging in the spring, breeds and produces adult V. prorsa progeny in the same summer. The progeny of these insects pass the winter as chrysalids and emerge the next spring as V.levana.

The levana form is characterized by a yellow and black pattern on the upper side of the wings while the prorsa form has black wings with a broad white transverse band. The lower surfaces differ only slightly. Dorfmeister proved that these variations are dependent on temperature. By the application of warmth to the pupae he produced prorsa out of the offspring of levana and by application of cold he obtained from levana, not the pure levana form but one intermediate between it and prorsa. He therefore concluded that temperature exerts its greatest influence during the change from larval into pupal stage or shortly afterwards. Weismann noticed that in V. prorsa-levana, temperature is effective only at the beginning of the pupal stage.

Upon the higher animals, temperature probably acts, but seldom as a direct cause of variation. The white coat which many quadrupeds develop on the approach of winter in northern and arctic climates is probably in great part a seasonally adaptive change; but it may also be to a certain extent the immediate or indirect response to cold. Sir J. Ross found that when a Hudson's Bay Lemming was protected from low temperature by keeping it in a cabin it retained its summer coat through the winter. On exposing it to 30° below zero the fur on the cheeks and a patch on each shoulder became perfectly white during the first night. It turned slowly white until at the end of a week it was entirely white with the exception of a dark band across the shoulders and down the middle of the back. No further change took place and the animal died of cold a few days later. Examination of the fur showed that only thetips of the hairs had become white.

A similar study was made by F. H. Welch who observed that during November, the American Hare (Lepus Americanus) grew stiff white hairs on the sides and back. These were easily distinguishable from the autumnal hairs which were turning white gradually, in that they were invariably white throughout. We have, therefore, a new crop of white hairs of gradual growth, or a blastogenic variation, developed under the stress of cold, and a rapid and direct transmutation of parts of the dark hairs to white; i. e. a somatic modification.

(b) Effect of Temperatures on Plants.

According to Ganong (1913) most of our plants do not grow in a temperature much below 40°F; their optimum is at about 85° but theywill hardly grow at all at 100°F. The reasons for growth depend upon a number of chemical and physical processes which are kept in orderly coöperation by the protoplasm. All of these processes are promoted by higher temperature, which fact explains the more rapid growth up to the optimum point. As the temperature rises to degrees higher than those to which the plant is accustomed, the processes get beyond the control of the protoplasm, thus injuring and finally destroying the coördination and stopping the growth. Another cause operatingto stop the growth is the commencement of injurious chemical reactions under higher temperature and the accumulation of waste products faster than they can be removed.

Production of chlorophyll is also dependent upon temperature (Livingston). Medium temperatures are most favorable and no greening occurs at very low or very high temperatures; photosynthesis, however, is only very slightly affected by temperature. The lower the temperature the more slowly does plasmolysis occur; the return of turgidity is more rapid as the temperature of the water is raised. The rate at which dissolved substances diffuse through protoplasm is increased with rise of temperature. Starch hydrolysis by diastase is hastened with higher temperatures up to 45° or 50°C.

It is supposed that organic acids (in cell sap) arise through incomplete oxidation of carbohydrates, the acid content being lower at higher temperatures. It has been shown that changes in

temperature, other conditions being equal, are sufficient to produce difference in the external appearance of certain plants and an instance is given in which a plant, which grows vertically upward at ordinary temperatures, bends or even assumes a horizontal position at lower temperatures.

Bauer (1911) cites a case of Primula sinosis which under ordinary conditions produces red flowers. If a plant is subjected to 30°-35°C a few weeks before blooming, the flowers will be white. If they are returned to 15°-20°C the buds opening immediately will still be white, but those developing later will again be red. In this case he points out that production of white flowers cannot be said to be inherited, but the capacity to produce white flowers at 30°C and red flowers at 15°C is the inherited quality.

III. Thermal Death Points.

(a) Theoretical.

Although there is no theoretical death point, i. e., a temperature at which an organism will always die, it has been found that under given conditions the fatal temperature is fairly constant.

Chick (1908) states that the rate of disinfection is influenced by temperature in an orderly manner so that the equation of Arrhenius can be applied. She also states (1909) that disinfection, in this case heat, is a process closely analagous to a chemical reaction, the disinfectant representing one reagent and the protoplasm the other. The process is found to proceed in accordance with the mass law, the number of surviving bacteria being substituted for the concentration of the reacting substance. The number of living bacteria when enumerated after successive intervals of time is found to decrease in a logarithmic manner. Miss Chick's experiments were carried out with vegetative and spore-bearing types of various bacteria using metallic salts, phenol, and coal tar derivatives and heat as the disinfecting agents.

Again in 1910, Chick found that disinfection by hot water is closely parallel to heat coagulation of protein. Both are consistent time processes and proceed in accordance with the Mass Law. Disinfection by this method is increased by the addition of minute amounts of acid. She studied the heat coagulation of proteins with solutions of crystallin hemoglobin and crystallin egg albumin. The heat coagulation was found to be a reaction between protein and water and the heat merely accelerates it; dry proteins are

still soluble after being heated at 110° to 130°. The addition of 4 cc. of 0.1N. alkali to 1 gram of egg albumin crystals in solution reduces the reaction rate to 1/60.

(b) Significance of Thermal Death Points.

Recent experiments show that heat does not kill bacteria instantaneously, but that we have an orderly process. This can be observed only within a very narrow range of temperature since the death rate rises rapidly with increase in temperature. Ten degrees rise in temperature may increase the death rate ten to one hundred times and death is almost instantaneous. It is customary to define the thermal death point as the temperature at which the organism will be killed in ten minutes. Thermal death point, however, does not depend upon species and temperature only, but varies with the age of the culture since older cells are less resistant than younger ones, especially if they are heated in their own products. Foods which have their flavor spoiled by too long heating can be sterilized by the discontinuous method, thus all spores are destroyed without injuring the food. (Marshall)

Buchanan states that the factors influencing the thermal death point are (1) time of exposure (that used for most thermal death point determinations is 10 minutes); (2) amount of moisture present (moist heat being more efficient than dry heat, due to the difference in the coagulation temperature of moist and dry proteins); (3) reaction and composition of the medium (acid fruits being easier to sterilize than neutral vegetables); (4) the presence of spores indicates two thermal death points, one for the spore and the other for the vegetative cell; and (5) specific character of the organism.

Ayers (1914) studied the effect of heat upon streptococci found in milk and cream, as well as those taken from the udder, feces and mouth of the cow. He found that 64.03% lived at 60°C

while all were destroyed at 73.9°C when heated for 30 minutes under conditions similar to pasteurization.

That time is an important factor in the death of bacteria is shown by Ayers and Johnson (1913) in their studies on pasteurization. They observed that 42% of the milk plants employing the flash method use temperatures too low to be efficient, while only 1.3% of those using the holder method are inefficient. They also observed that a heating period of three hours causes a marked decrease in bacteria over a one-half hour heating period at 54.4°C or 57.2°C; while a six-hour period at 62.8°C does not produce any more destruction than a one-half hour period. They found (1915) that there is considerable variation in the thermal death points of 174 cultures of colon bacilli isolated from cow feces, milk and cream, human feces, flies and cheese, when heated under conditions similar to pasteurization for thirty minutes. At 60°C, 54.59% survived while one culture was not destroyed at 65.8°C on the first heating, but in repeated experiments it was always destroyed. Although there is a difference of only 2.8°C, 87.3% of those which survived 60°C died at 62.8°C. It seems evident that 62.8°C for thirty minutes is a critical temperature for B. coli, but its use as an index of efficiency in pasteurization is complicated by the ability of some strains to survive a temperature of 62.8°C for thirty minutes.

The effect on the organisms of the medium in which they are suspended is shown by Bartlett and Kinne (1913). They found that Staphylococcus aureus is quickly killed in glycerine, water, olive oil, cottonseed oil, and paraffin for different periods at the

temperature of boiling water. Spores of anthrax and B. subtilis die in boiling water usually in three minutes or less. In glycerine and oil they are found to live even when heated to the boiling point for 75 and 50 minutes, respectively. Similarly in the autoclave these spores live in oil fifteen minutes and in glycerin ten minutes under a pressure of 7 1/2 pounds. In water they do not live over five minutes at this pressure. B. vitalis is found to be more resistant in glycerine and oil than in water.

Russell and Hastings (1902) observed that when bacteria were heated in milk exposed to the air, they withstood a higher temperature than when air was excluded. In their experiments they found that milk drawn from below the surface was sterile while there were living bacteria in the surface membrane. A second membrane was allowed to form and proved to be sterile. Two explanations for this phenomenon are given : (1) the surface temperature is lower than the rest; and (2) the bacteria are protected by the membrane. The first of these explanations was disproved by putting the membrane into water of the same temperature. In this same line, Brown and Peiser (1916) concluded that the casein and fat in milk offer some protection to lactic acid bacteria but in their experiments with cotton plugged tubes, they found thatmilk, unprotected, gave the same results as that with paraffin oil on the surface and in Sternberg bulbs. They also observed that the addition of small amounts of lactic acid did not change the thermal death point.

It is the opinion of Jordan (1910) that the Sternb erg bulbs offer the best test for thermal death points. He states that while tubercle bacilli in suspension in milk are destroyed at 60°C in 15 or 20 minutes the pellicle may contain living organisms after 60 minutes.

IV. Previous Work on Thermal Death Points of Yeasts.

Experiments for determining the action of heat on a number of yeasts under varying conditions were carried on by Kayser (1889) and the conclusion was reached that yeasts are more resistant in the dry than in the moist state, but that the limits in dry heat are larger than in moist heat because of the uncertain temperature. He also observed that spores resist higher temperatures than the vegetative cells and in the same order as the corresponding yeasts; that the cells are more resistant if dried at low, than at high temperatures; that young cells are more resistant than old ones; and that heredity is an important factor i. e. that those yeasts which have passed thru the spore stage are more resistant than those which have not.

Cochran and Perkins (1914) tested yeasts in sugar solutions of varying densities and found that they died more quickly in the more dense solutions.

Wells (1917) investigated bread and in twenty trials found yeast three times in bread baked at 65°C and twice found none. Living cells were found in well controlled bread baked at 66°C, but none were found in bread baked at 68°. Presumably, therefore, 68°C is approximately the thermal death point in bread.

Yeast cells usually do not withstand more than 50°C to 60°C moist heat (Klöcker). Hansen found that strong young cells of Saccharomyces ellipsoideus II, die after five minutes heating in distilled water between 54° and 56°C and that old cells under the same conditions withstand 60°C. Quite ripe spores of the same species, partially dried in a gypsum block, withstand 62°C but not 66°C.

Similar experiments with Saccharomyces cerevisiae I, show that strong young cells resist 52°C but not 54°C; and the spores treated in the same way as the previous species resist 58°C but not 62°.

That the thermal death point varies with the strain is shown by the following: Kayser (1890) found that Saccharomyces mali duclauxi is killed at 55°C; Johnson (1905), that Saccharomyces thermantitonum is able to resist high temperatures, the highest for budding being 84°C; Will (1907-'08) that the thermal death point of various yeasts varies between 60° and 65°C; Owen (1913) that the thermal death point of Saccharomyces Zopfi is 90°C; Admetz (1889) that dry cells of Saccharomyces lactis die at 50°-60°C and moist ones at 56°C and Bay (1893) that Saccharomyces Willianus dies at 70°C.

In studies of the lactose fermenting yeast producing foamy cream, Hunter (1917) found that the optimum temperature was near 37°C and the thermal death point near 55°C for ten minutes. Hunziker (1914) states that the thermal death point of all forms of yeast found in a large number of investigations of fermented condensed milk was below 180°F.

Zavella observed that yeasts found in leaky cans do not grow after being heated at 140°F and incubated at 29°-33°C; others do not grow after being heated at 212°F and incubated. Oidium, Torula and Mycoderma vini all die at low temperatures while S. ellipsoideus is not killed at 122°F for ten minutes but is killed at 199.5°F for ten minutes.

V. Experimental.

Very little attention seems to have been given to the technique and apparatus which may be used for determining the thermal death points for organisms. The Society of American Bacteriologists has specified that the organism shall be held for ten minutes at the various temperatures in plain broth. Such instructions are quite inadequate. Undoubtedly the proposals of Bigelow and Esty should be given serious consideration on account of their experience in such work. Their method was devised for the study of microörganisms which survive the sterilization process in the canning industry. Since this has not been described elsewhere in this paper, it is proper to give it here. Bigelow and Esty state it as follows:

"Cultures of the organism to be tested were grown on nutrient agar slants until a luxuriant growth occurred along the inoculated area (about 48 hrs.) This growth was then inoculated into nutrient broth of P_H 7.0 and incubated at the optimum temperature of that specific organism for one week. One c.c. of this suspension was inoculated into 10 c.c. of the medium to be used and heated to 85°C fifteen minutes to kill all vegetative forms. Special culture tubes, 7 m.m. (inside diam.) x 250 m.m. long and 1 m.m. thickness of wall, were sterilized and then inoculated with a suspension of spores of these organisms."

"The tubes were then sealed off to within two inches of the surface of the liquid, placed in a Wasserman rack and held temporarily in an ice bath until heated. The rack of sealed tubes was transferred to the oil bath, adjusted to the desired temperature, and subjected to this temperature for different lengths

of time. Upon removal from the bath, the tubes were cooled in ice water and held in an ice box until determination could be made on the sterility of these cultures after heating. The initial count was made according to Standard Methods, and the hydrogen ion concentration of the medium was determined colorimetrically."

Since the data for which Bigelow and Esty are searching, is the longevity of microörganisms in hermetically sealed containers, their determinations were made in sealed tubes. For the purpose of this investigation such technique was deemed unnecessary.

The cultures of yeasts used in this work were supplied from the collection of the Division of Bacteriology of the University of Illinois. Careful tests for purity of culture including cultivation in two per cent tartrate broth were carried out before the yeasts were subjected to any of the experiments reported herewith. A list of the yeasts is given below:-

1. Saccharomyces glutinis (Fresenius) Cohn.
2. Zygosaccharomyces bisporus Anderson.
3. Saccharomyces anomalous Anderson.
4. Willia Belgica Lindner.
5. Mycoderma lactis.
6. Saccharomyces albus.
7. Mycoderma vini.
8. Cryptococcus glabratus Anderson.
9. Parasaccharomyces Thomasii Anderson.
10. Saccharomyces ellipsoideus.
11. Torula datilla.
12. Mycoderma monosa Anderson.
13. Cryptococcus aggregatus Anderson.

14. Burgundy Wine yeast.

15. Saccharomyces Curtis.

16. Saccharomyces Binsh.

17. Saccharomyces hominis Busse.

18. Brewers yeast.

19. Oidium albicans Ch. Robin.

20. Torula humicola Daczewska.

21. Saccharomyces anomalous Bioletti.

22. Torula monosa.

23. Mycoderma rugosa Anderson.

24. Cryptococcus Ludwigi.

Each organism to be tested was transplanted from glucose agar slant into glucose broth and grown at 37°C for 12 to 24 hours in order to insure actively growing cells, 0.5 cc. of this suspension was then put into a tube containing about 10 cc. of the sterile media.

In these tests a jacketed water-bath was used to insure a constant temperature. Jordan believes that the tests for thermal death points are best made by the Sternberg bulb which can be sealed and completely immersed into the water-bath but Brown and Peiser have found that the results in cotton plugged tubes are very similar to those in the bulbs.

The tubes in which these tests were made were 5 x 5/8 inches and of as thin glass as possible. They were placed in the water so that the level of the media was well below that of the water, and one thermometer was kept in the water-bath and one in a test-tube under the same conditions as the organisms. Each series was started at a moderate temperature which was not allowed to vary

over 0.5°C, and after 10 minutes exposure the tubes were removed, each organism was plated on glucose agar, incubated at 37°C, and observed for at least 5 days. If no colonies appeared in this time it was assumed that the yeast had died. The same tubes were put in at a temperature of 3°C higher, and the experiment repeated every three degrees until it was certain that all would have died. To lessen the experimental error there were never more than 10 tubes in the bath at the same time. The plates on which the yeasts were grown were sterilized in the autoclave after pouring them in order to diminish the danger of contamination from the air. Eijkman points out a possible error in using the plate method of determining death of organisms because so many cells are damaged by heat that they develop slowly but are not killed. He describes one experiment with B. coli, in which no colonies were visible on gelatin in three days of incubation but after fifteen days there were 670,000 colonies, so he believes that the cultures should be made in fluid media.

In order to get an approximate idea with regard to the temperature relations, the maximum temperatures at which the yeasts could grow were determined.

Twenty-four representative yeasts were selected and each of these was inoculated from glucose agar slants into glucose broth and glucose agar slants. These cultures were incubated for 24 hours at temperatures between 40° and 55°C. Each day new cultures were tested in a temperature 3° higher than the preceding day, and each organism was eliminated from the test as soon as it was found at what temperature its growth was inhibited. (Table I.)

Table I.

Maximum Temperature for Growth.

		Temperature.					
		40°C	43°	46°	49°	52°	55°
1.	Saccharomyces glutinis	0	0	0	-	-	-
2.	Zygosaccharomyces bisporus	+	+	0	-	-	-
3.	Saccharomyces anomalous (Anderson)	0	0	0	-	-	-
4.	Willia belgica	+	0	0	-	-	-
5.	Mycoderma lactis	+	+	+	0	-	-
6.	Saccharomyces albus	-	-	-	-	-	-
7.	Mycoderma vini	+	+	+	0	-	-
8.	Cryptococcus glabratus	+	+	+	-	-	-
9.	Parasaccharomyces Thomasii	+	0	0	0	-	-
10.	Saccharomyces ellipsoideus	0	0	0	-	-	-
11.	Torula datilla	0	0	0	-	-	-
12.	Mycoderma monosa	+	+	+	-	-	-
13.	Cryptococcus aggregatus	0	0	-	-	-	-
14.	Burgundy Wine Yeast	0	0	-	-	-	-
15.	Saccharomyces of Curtis	0	0	-	-	-	-
16.	Saccharomyces of Binot	0	0	-	-	-	-
17.	Saccharomyces hominis	-	-	-	-	-	-
18.	Brewers Yeast	+	+	+	0	0	-
19.	Oidium albicans	+	+	+	-	-	-
20.	Torula humicola	-	-	-	-	-	-
21.	Saccharomyces anomalous(Bioletti)	0	0	0	-	-	-
22.	Torula monosa	0	0	0	0	-	-
23.	Mycoderma rugosa	+	+	+	0	0	0
24.	Crytococcus Ludwigi	-	-	-	-	-	-

\+ = growth
\- = no growth
0 = slight growth

The data indicated in Table I. shows that none of the yeasts examined possess ability to develop at very high temperatures. The great majority ofthem failed to grow above from 43° to 46°C. Oidium albicans and Mycoderma rugosa, Anderson were among those which grew at temperatures above 46°C, the latter growing at 55°C.

The first experiment was tried with the organisms suspended in glucose broth. It will be noticed that the thermal death point of Saccharomyces ellipsoideus in these experiments was found to be 52°C, while Hansen found that Saccharomyces ellipsoideus II, dies in water between 54° and 56°C in five minutes and Zavella believes that it is not killed at 50°C, but does not live at 93°C. The above variation is probably due in part to the difference in strains of the yeast used.

The three yeasts showing high resistance to heat were carried through the temperature of boiling water (approximately 98°C.) for 30 minutes both in the glucose broth and milk. (Table II).

Table II.

Thermal Death Points.

Glucose Broth Suspension for 10 Minutes.

	40°	43°	46°	49°	52°	55°	58°	61°	64°	90°(boiling temp.) of water.	10'	20'	30'
1. Saccharomyces Glutinis	+	+	+	+	+	+	-	-	-				
2. Zygosaccharomyces bisporus	+	+	+	+	+	+	-	-	-				
3. Saccharomyces anomalous(Anderson)	+	+	+	±	-	-	-	-	-				
4. Willia belgica	+	+	+	+	+	+	+	+	+	+	-	-	-
5. Mycoderma lactis	+	+	+	+	+	+	-	-	-				
6. Saccharomyces albus	+	+	+	+	-	-	-	-	-				
7. Mycoderma vini	+	+	+	+	+	+	+	-	-				
8. Cryptococcus glabratus	+	+	+	+	+	+	-	-	-				
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+	-	-
10. Saccharomyces ellipsoideus	+	+	+	+	-	-	-	-	-				
11. Torula datilla	+	+	+	+	-	-	-	-	-				
12. Mycoderma monosa	+	+	+	+	+	+	+	-	-				
13. Cryptococcus aggregatus	+	+	+	-	-	-	-	-	-				
14. Burgundy Wine Yeast	+	+	+	-	-	-	-	-	-				
15. Saccharomyces of Curtis	+	+	+	-	-	-	-	-	-				
16. Saccharomyces of Binot	+	+	+	+	-	-	-	-	-				
17. Saccharomyces hominis	+	+	-	-	-	-	-	-	-				
18. Brewers Yeast	+	+	+	+	+	+	+	-	-				
19. Oidium albicans	+	+	+	+	+	+	-	-	-				
20. Torula humicola	+	+	-	-	-	-	-	-	-				
21. Saccharomyces anomalous(Bioletti)	+	+	+	+	+	-	-	-	-				
22. Torula monosa	+	+	+	+	+	+	+	+	+	+	+	-	-
23. Mycldderma rugosa	+	+	+	+	+	+	-	-	-				
24. Cryptococcus Ludwigi	+	+	+	+	+	+	-	-	-				

\+ = growth
\- = no growth.

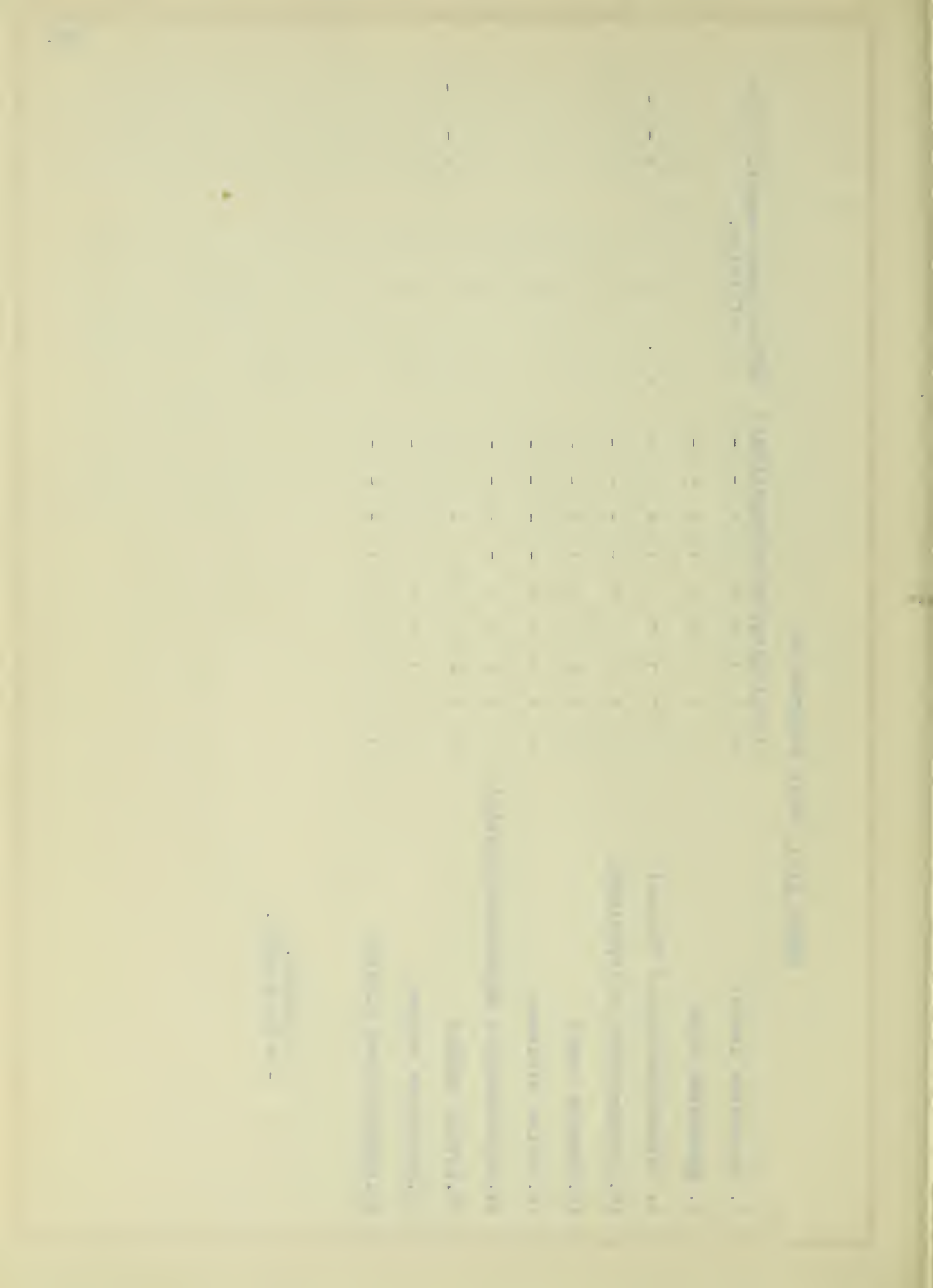
Before continuing, the number of organisms was cut down to twelve to eliminate the repetition necessary when so many were used, for it was thought best not to have more than twelve tubes in the water at one time.

The experiment with milk was carried on under the same conditions as the previous experiment and no special effort was made to exclude the air. Russell and Hastings, and Brown and Peiser, state that bacteria heated in milk which is exposed to air have higher resistance, but in this case no pellicle was observed and since the thermal death points in milk were very close to that in dextrose broth, the pellicle is probably not an important factor in this experiment. (Table III).

Table III. Milk Suspension.

	40°	43°	46°	49°	52°	55°	58°	61°	64°	90° (boiling temp of water.)	10'	20'	30'
5. Mycoderma lactis	+	+	+	+	+	+	+	-	-				
7. Mycoderma vini	+	+	+	+	+	+	+	-	-				
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+	-	-
10. Saccharomyces ellipsoideus	+	+	+	+	+	-	-	-	-				
18. Brewers Yeast	+	+	+	+	+	+	+	-	-				
19. Oidium albicans	+	+	+	+	+	-	-	-	-				
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	-	-	-	-				
22. Torula monosa	+	+	+	+	+	+	+	+	+	+	+	-	-
23. Mycoderma rugosa	+	+	+	+	+	+	+	-	-				
24. Cryptococcus Ludwigi	+	+	+	+	+	+	-	-	-				

+ = growth.
- = no growth.



Suspended in milk the yeasts were able to survive 52°C but began to drop out above that temperature. Torula monosa was able to survive 64°C as was Parasaccharomyces Thomasii. The results secured with milk differ very little from those for glucose broth.

The number of organisms was further reduced to five. These were heated, under the same conditions as the other experiments, in distilled water, then in Hydrochloric Acid in varying dilutions, and finally in vinegar and the juice of home-canned, unsweetened gooseberries. Chick(1910) found that disinfection by hot water is influenced by the addition of minute amounts of acid and Buchanan states that acid fruits are more easily sterilized than neutral vegetables. In these experiments the thermal death points in acid seem to be only slightly lower than those in sugar and salt solutions, but they are the same as those in milk if the concentration of the acid is not too high. Vinegar seems to possess a very high germicidal action while gooseberry juice is less destructive. Table IV).

Table IV. Suspension in Distilled Water.

	Temperatures.								
	40°	43°	46°	49°	52°	55°	58°	61°	64°
7. Mycoderma vini	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous(Bioletti)	+	+	+	+	+	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	-	-	-	-
Suspension in 9 H_2O + 1 N/200 HCl.									
7. Mycoderma vini	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous(Bioletti)	+	+	+	+	+	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	-	-	-	-
Suspension in 8 H_2O + 2 N/200 HCl.									
7. Mycoderma vini	+	+	+	+	+	+	+	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	-	-
21. Saccharomyces anomalous(Bioletti)	+	+	+	+	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	-	-	-	-
Suspension in 7 H_2O + 3 N/200 HCl.									
7. Mycoderma vini	+	+	+	+	+	+	+	-	
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	
18. Brewers Yeast	+	+	+	+	+	+	+	-	
21. Saccharomyces anomalous(Bioletti)	+	+	+	+	+	-	-	-	
24. Cryptococcus Ludwigi	+	+	+	+	+	+	-	-	
Suspension in 5 H_2O + 5 N/200 HCl.									
7. Mycoderma vini	+	+	+	+	+	+	+	-	
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	
18. Brewers Yeast	+	+	+	+	+	+	+	-	
21. Saccharomyces anomalous(Bioletti)	+	+	+	-	-	-	-	-	
24. Cryptococcus Ludwigi	+	+	+	+	+	-	-	-	
Suspension in 3 H_2O + 7 N/200 HCl.									
7. Mycoderma vini	+	+	+	+	+	+	+	-	
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	
18. Brewers Yeast	+	+	+	+	+	+	+	-	
21. Saccharomyces anomalous(Bioletti)	+	+	+	-	-	-	-	-	
24. Cryptococcus Ludwigi	+	+	+	+	-	-	-	-	

+ = growth.
- = no growth.

Table IV. (continued).

Suspension in 9 H_2O + 1 N/20 HCl.

	Temperature. 40°	43°	46°	49°	52°	55°	58°	61°
7. Mycoderma vini	+	+	+	+	-	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	-	-	-	-	-	-
24. Cryptococcus Ludwigi	-	-	-	-	-	-	-	-

Suspension in 8 H_2O + 2 N/20 HCl.

7. Mycoderma vini	+	+	+	+	+	+	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	-	-	-	-	-
24. Cryptococcus Ludwigi	-	-	-	-	-	-	-	-

Suspension in 7 H_2O + 3 N/20 HCl.

7. Mycoderma vini	+	+	+	+	-	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	-	-	-	-	-
24. Cryptococcus Ludwigi	-	-	-	-	-	-	-	-

Suspension in 5 H_2O + 5 N/20 HCl.

7. Mycoderma vini	+	+	+	+	+	+	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	-	-	-	-	-
24. Cryptococcus Ludwigi	-	-	-	-	-	-	-	-

Suspension in 3 H_2O + 7 N/20 HCl.

7. Mycoderma vini	+	+	+	+	-	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	-	-	-	-	-	-
24. Cryptococcus Ludwigi	-	-	-	-	-	-	-	-

\+ = growth.
\- = no growth.

Table IV.(continued).

Suspension in N/20 HCl.

	Temperature. 40°	43°	46°	49°	52°	55°	58°	61°
7. Mycoderma vini	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+
18.Brewers Yeast	+	+	+	+	+	+	-	-
24.Cryptococcus Ludwigi	-	-	-	-	-	-	-	-
21.Saccharomyces anomalous(Bioletti)	+	-	-	-	-	-	-	-

Suspension in Vinegar.

7. Mycoderma vini	-	-	-	-	-	-	-	-
9. Parasaccharomyces Thomasii	+	-	-	-	-	-	-	-
18.Brewers Yeast	-	-	-	-	-	-	-	-
21.Saccharomyces anomalous(Bioletti)	-	-	-	-	-	-	-	-
24.Cryptococcus Ludwigi	-	-	-	-	-	-	-	-

Suspension in Gooseberry Juice.

7. Mycoderma vini	+	+	+	+	+	+	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	-	-	-
18.Brewers Yeast	+	+	+	+	+	+	+	-
21.Saccharomyces anomalous(Bioletti)	+	+	+	+	-	-	-	-
24.Cryptococcus Ludwigi	+	-	-	-	-	-	-	-

\+ = growth.
\- = no growth.

The tests were next tried with saccharose in varying concentrations. These solutions were made by weighing the sugar and calling 5, 10, 15, etc. grams to 100 cc. of water 5%, 10%, 15% etc. Cochran and Perkins (1914) found that yeast died more quickly in the more dense sugar solutions but in these experiments it was found that the variation of sugar concentration from 5 to 60% makes very little difference in the thermal death point(Table V).

Table V.

Suspension in 5% Sugar.

	Temperature										
	40°	43°	46°	49°	52°	55°	58°	61°	64°	67°	70°
7. Mycoderma vini	+	+	+	+	+	+	+	+	+	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	-	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	-	-	-	-	-	-	-

Suspension in 10% Sugar.

7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	-	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	-	-	-	-	-	-

Suspension in 15% Sugar.

7. Mycoderma vini	+	+	+	+	+	+	-	-	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	-	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	-	-	-	-	-	-	-

Suspension in 20% Sugar.

7. Mycoderma vini	+	+	+	+	+	+	+	-	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	+	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	+	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	-	-	-	-	-	-	-

Suspension in 30% Sugar.

7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	+	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	-	-	-	-	-	-

+ = growth.
- = no growth.

Table V.(continued).

	40°	43°	46°	49°	52°	55°	58°	61°	64°	67°	70°
Suspension in 40% Sugar. Temperature.											
7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18.Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21.Saccharomyces anomalous(Bioletti)	+	+	+	+	+	-	-	-	-	-	-
24.Cryptococcus Ludwigi	+	+	+	+	+	+	-	-	-	-	-
Suspension in 50% Sugar.											
7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18.Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21.Saccharomyces anomalous(Bioletti)	+	+	+	+	+	+	-	-	-	-	-
24.Cryptococcus Ludwigi	+	+	+	+	+	+	-	-	-	-	-
Suspension in 60% Sugar.											
7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18.Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21.Saccharomyces anomalous(Bioletti)	+	+	+	+	+	+	-	-	-	-	-
24.Cryptococcus Ludwigi	+	+	+	+	+	+	-	-	-	-	-

\+ = growth.
\- = no growth.

As shown by these experiments, sugar solutions of increasing concentration had little effect. Such data were not expected since it was believed that a combination of high temperature and concentrated solutions would prove toxic to the yeasts.

The salt (NaCl) solutions were prepared in the same way as the sugar solutions. Although it was supposed that salt would have some germicidal action it was found that the yeasts have about the same thermal death point in salt solutions as they have in sugar. Table VI)

Table VI.

	Temperature. 40°	43°	46°	49°	52°	55°	58°	61°	64°	67°	70°
Suspension in 5% Salt Solution.											
7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	+	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	+	-	-	-	-	-
Suspension in 10% Salt Solution.											
7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	+	+	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	+	-	-	-	-	-
Suspension in 15% Salt Solution.											
7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	+	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	+	+	+	-	-	-
Suspension in 20% Salt Solution.											
7. Mycoderma vini	+	+	+	+	+	+	+	+	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	-	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	-	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	+	+	+	-	-	-
Suspension in 30% Salt Solution.											
7. Mycoderma vini	+	+	+	+	+	+	+	-	-	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	+	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	-	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	+	+	+	-	-	-
Suspension in Saturated Salt Solution.											
7. Mycoderma vini	+	+	+	+	+	+	+	+	+	-	-
9. Parasaccharomyces Thomasii	+	+	+	+	+	+	+	+	+	+	+
18. Brewers Yeast	+	+	+	+	+	+	+	+	+	-	-
21. Saccharomyces anomalous (Bioletti)	+	+	+	+	+	-	-	-	-	-	-
24. Cryptococcus Ludwigi	+	+	+	+	+	+	+	+	-	-	-

\+ = growth.
\- = no growth.

These organisms were also tested with physiological salt solution in Sternberg bulbs with the same results which were obtained with milk and glucose broth in the cotton plugged tubes.

Marshall (1917) states that heat does not kill bacteria instantaneously, but death takes place in an orderly manner which can only be observed in a very narrow range of temperature, because the death rate rises rapidly with increase in temperature. Mycoderma vini and Brewers Yeast have been examined in physiological salt solution to estimate the variation in length of life with an increase or decrease of temperature from the thermal death point. This point was found to be 60°C for both yeasts. At a temperature of 65°C both yeasts died in less than five minutes while at 55°C--only five degrees below the thermal death point--they resisted for over four hours.

VI. Summary.

1. Instructions for experiments on thermal death points are not clear, the only standard limitation being that given by the American Society of Bacteriologists.

2. Some investigators approve of the use of Sternberg bulbs, but the results of these experiments show the death points to be the same in the bulbs as in the tubes. In instances where the death point in sealed containers was desired these would probably have a great advantage as would the method of Bigelow and Esty.

3. Russell and Hastings, and Brown and Peiser believe that the resistance of bacteria is increased in milk heated in contact with the air. In these experiments no pellicle was observed and the death point was what was expected from the results of other experiments.

4. Although Chick (1910) and Buchanan state that the addition of small amounts of acid hastens the process of disinfection by heat, the yeasts seem to present a different problem, for to them small amounts of acid seem favorable instead of hastening their death.

5. Hunter (1920) describes a yeast which is responsible for spoilage of oysters. This yeast is similar in appearance and temperature relations to the pink yeast used in these experiments--Cryptococcus Ludwigi.

VII. Conclusions.

1. There is no single thermal death point for yeasts, but each yeast has its own critical temperature.

2. The death point in vinegar was lower than in any of the other solutions.

3. The Sternberg bulbs seem to have had no advantage over the cotton plugged tubes.

4. Concentrated solutions of sugar and salt are advantageous rather than germicidal to the yeasts.

5. Acids present the most unfavorable conditions.

6. In the standard media the non-spore bearing yeasts die between 52°C and 58°C while the spore bearing yeasts resist the temperature of boiling water for ten minutes.

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Studies of thermal death points of yeasts

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